Supplementary Discussion

Acute stress and chronic stress

There are different methods to model stress responses in mice. Many are effective in modelling acute, short-term stress[1-3]. However, because mice can habituate to one single stressor after a few times, modelling chronic, long-lasting stress is much more challenging. Currently, the most robust method that has been widely validated and accepted to model chronic stress at behavioural, circuitry, and physiological levels is the model utilized in this study—chronic unpredictable stress model (also called chronic variable stress)[4-9]. By altering stressors each day in a randomized fashion, mice do not habituate to a single stressor, allowing this model to be applied long-term to study chronic stress.

Systemic factors vs. niche factors of stem cell activity

Proliferation of *Drosophila* germline stem cells is under direct control of insulin and the steroid hormone twenty-hydroxyecdysone (20E)[10,11]. In mammals, haematopoietic stem cell maintenance is influenced by oestrogen and liver-derived thrombopoietin[12-14]. These examples demonstrate how systemic hormones can act on stem cells directly to alter their behaviours. Here, we show a distinct example in which a systemic factor (corticosterone) acts on the niche (DP) to regulate HFSCs.

DP is a key niche cell type that tunes the ability of HFSCs to transition from quiescence to activation[15-19]. We show here that *Gas6* expression in DP is kept at a low level by circulating corticosterone, providing an example of an activating niche factor under suppression by a systemic regulator. The corticosterone-GAS6-AXL axis adds another layer of regulation on top of other known niche factors, extending or shortening telogen based on the overall physiological state of the organism (Extended Data Fig. 10g). Distinct from other local niche factors whose levels fluctuate at different telogen phases, corticosterone remains relatively constant and only becomes up-regulated under stress or as animals age. These increases in corticosterone

counteract local activating signals and lower *Gas6* levels, leading to significantly extended telogen (Extended Data Fig. 10g). As corticosterone levels display a diurnal rhythm and are subject to seasonal changes[20,21], it is also possible that the corticosterone-GAS6 axis helps fine tune activity of HFSCs based on circadian rhythm[22-24] or contributes to regulating seasonal moulting in wild animals.

Repeated entry into the hair cycle without exhaustion

Quiescence has been postulated to preserve the ability of stem cells to regenerate tissues long-term[25-30]. Here, we identified a pathway through which HFSCs can enter substantially more rounds of anagen throughout life without losing their regenerative potential. Our results also suggest that without corticosterone, the regenerative capacity of HFSCs does not decline substantially with age, as we found that old ADX animals regenerate hair follicles at a frequency faster than young control animals. Stem cell quiescence is known to prevent tumour initiation by HFSCs carrying active oncogenes or inactive tumour suppressors[31]. However, we did not observe apparent signs of hyperplasia in our ADX mice or *Gas6* overexpression mice, suggesting that, in the absence of tumour-associated mutations, loss of HFSC quiescence due to modulations of the corticosterone-GAS6-AXL axis does not automatically lead to aberrant overgrowth.

In conclusion, our study showcases the remarkable potential of HFSCs when released from the systemic control of corticosterone. Our findings also open the door to future investigations into corticosterone-mediated regulation of stem cell quiescence in other organ systems, as well as potential therapeutic strategies to combat the detrimental impact of stress.

Supplementary References

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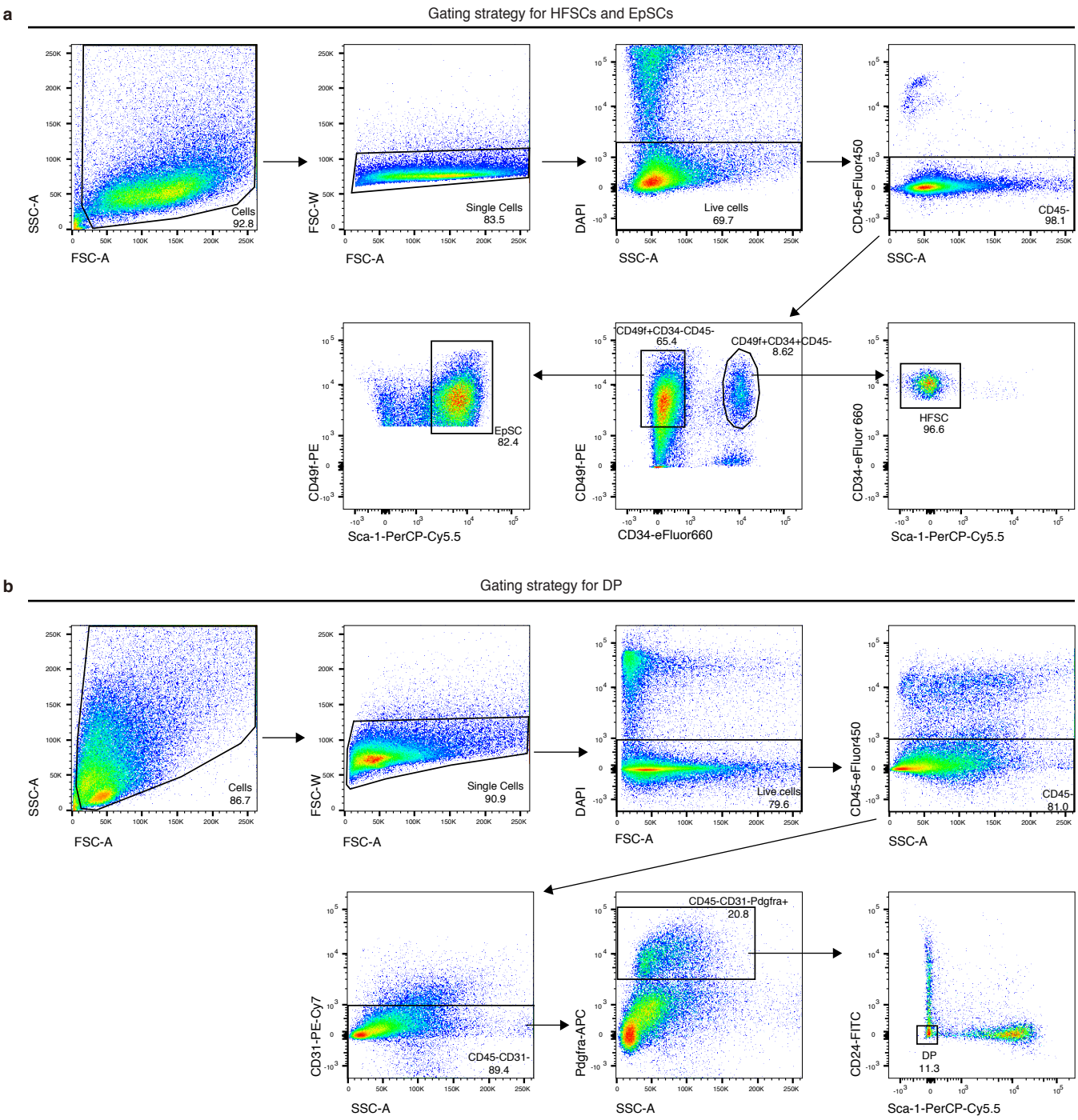

Supplementary Figure 1. Gating strategies used for sorting and FACS analysis of HFSCs, EpSCs or DP cells. a, Gating strategies to sort hair follicle stem cells (HFSCs) (CD45-, CD49f+, CD34+, Sca-1-) and epidermal stem cells (EpSCs) (CD45-, CD49f+, CD34-, Sca-1+). The gating strategy was used in RNAseq analysis (Fig. 3f, Extended Data Fig. 6a-d), qRT-PCR analysis (Extended Data Fig. 4a, 6e,f, 7a-d, 8e, 9h,j) and FACS analysis (Extended Data Fig. 2c). **b**, Gating strategies to sort dermal papilla (DP) cells (CD45-, CD31-, Pdgfra+, CD24-, Sca-1-). The gating strategy was used in RNAseq analysis (Extended Data Fig. 7e,h-j) and qRT-PCR analysis (Fig. 3a, Extended Data Fig. 9b, 10b).